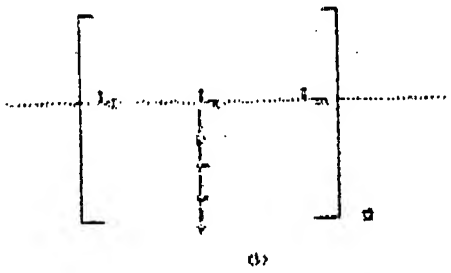
AMENDMENT TO THE CLAIMS

Claim 1 (currently amended): A method of preparing covalent antibodies that ~~form complexes with~~ that bind a ~~poly~~peptide or protein covalently ~~antigens wherein said complexes do not dissociate on treatment with a protein denaturant,~~ and catalytic antibodies that covalently bind to and hydrolyze the peptide or protein ~~to catalyze cleavage of peptide bonds in a polypeptide antigen~~, comprising:

producing in an organism, antibodies to a covalently reactive polypeptide antigen analogue (pCRA) of formula (1):



(1)

wherein, $L_1 \ldots Lx \ldots Lm$ are components defining [[an]] antigenic determinant of the peptide or protein,

Lx is an amino acid residue,

L' is a side chain functional group of Lx,

Y" is ~~atom, covalent bond~~ or a linker,

Y' is an optional charged or neutral group,

Y is a covalently reactive electrophilic group that reacts specifically with an antibody that binds to said antigenic determinant,

optionally, Y", Y' or Y contains a water-binding group as a terminal or internal component;

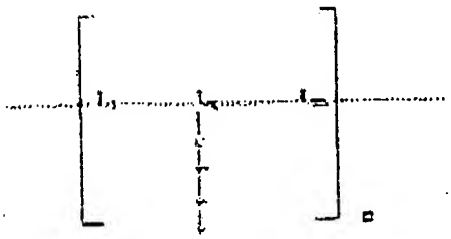
n is an integer from 1 to 1000; and

m is from 4 to 30;

screening and selecting for antibodies that covalently bind to the pCRA or to the peptide or protein having one or more of the antigenic determinant comprising the pCRA to identify covalent antibodies produced in the organism; and

screening and selecting for antibodies that covalently bind to the pCRA and screening from among the covalently binding antibodies for antibodies that catalytically hydrolyze a peptide bond in the peptide or protein having the antigenic determinant comprising the pCRA to identify catalytic antibodies produced in the organism, thereby preparing covalent antibodies and catalytic antibodies.

Claim 2 (withdrawn and currently amended): A water-binding, covalently reactive polypeptide antigen analogue (pCRAW) of formula (1):



(I)

wherein, L_1 . . . Lx . . . Lm are components defining an a polypeptide antigenic determinant of the polypeptide or protein,

Lx is an amino acid residue,

L' is a side chain functional group of Lx,

Y" is ~~atom, covalent bond~~ or a linker,

Y' is an optional charged or neutral group,

Y is a covalently reactive electrophilic group that reacts specifically with an antibody that binds to said antigenic determinant,

Y", Y' or Y contains a water-binding group as a terminal or internal component;

n is an integer from 1 to 1000; and

m is from 4 to 30.

Claim 3 (withdrawn): The pCRAW of claim 2, wherein the water-binding group is composed of a site that binds a metal ion which chelates one or more water molecules.

Claim 4 (withdrawn): The pCRAW of claim 3, in which the metal is zinc, copper, nickel, cobalt, calcium or magnesium.

Claim 5 (withdrawn): The pCRAW of claim 2, in which the metal binding group is selected from: -(His).sub.n- where n=2 or more, -Cys-X-Cys-Cys- or -Cys-X-Cys- wherein X is an amino acid residue, ethylene diamine tetraacetic acid or diaminomethyl pyridine.

Claim 6 (currently amended): The method of claim 1, wherein binding of the covalent and catalytic antibodies to [[a]] the ~~polypeptide antigen~~ or the protein is resistant to dissociation by a denaturant that disrupts non-covalent antigen binding.

Claim 7 (currently amended): The method of claim 1, wherein the binding of the covalent and catalytic antibodies to [[a]] the ~~polypeptide antigen~~ or the protein is resistant to dissociation by 2% sodium dodecyl sulfate.

Claim 8 (currently amended): The method of claim 1, wherein the protein ~~polypeptide antigen~~ is HIV-1 gp120.

Claims 9-10 (canceled)

Claim 11 (currently amended): The method of claim 1, wherein the covalent antibodies or catalytic antibodies are polyclonal antibodies identified in the serum of said organism ~~by:~~

~~a) screening and selection for covalently binding antibodies; and~~

~~b) screening and selection for catalytic activity.~~

Claim 12 (currently amended): The method of claim 1, wherein the antibodies are monoclonal antibodies or antibody fragments obtained from lymphocytes of said organism; wherein the ~~by~~ steps of screening and selecting further comprise ~~comprising:~~

a) preparing a library of hybridoma cell lines, virus-transformed cell lines or immunoglobulin fragment genes expressed from a vector prior to screening and selecting for the covalent antibodies and the catalytic antibodies or antibody fragments thereof;

~~b) screening for covalent activity of antibodies or antibody fragments by their binding to an antigenic pCRA or a polypeptide;~~

~~c) screening for catalytic hydrolysis of [[a]] the polypeptide by the antibodies or antibody fragments of step a) and step b);~~ and

b) ~~d)~~ purifying the covalent antibodies and catalytic antibodies or the antibody fragments thereof.

Claim 13 (currently amended): The method of claim 1[[2]], in which the antigenic determinant of the pCRA is ~~the CRA derivative of~~ comprises gp120, VIP, Factor VIII, epidermal growth factor receptor, CD4, β-amyloid peptide 1-40, or β-amyloid peptide 1-42.

Claim 14 (canceled).

Claim 15 (currently amended): The method of claim 12, wherein the organism is a transgenic ~~transonic~~ mouse expressing human antibody genes.

Claim 16 (original): The method of claim 12, wherein the organism is a mouse.

Claim 17 (original): The method of claim 12, wherein the vector is selected from the group consisting of phage display vectors, retroviral display vectors, yeast display vectors, bacterial display vectors and mammalian display vectors.

Claim 18 (currently amended): The method of claim 1[[2]], wherein the antibody fragments are single chain Fv fragments obtained ~~expressing covalent or catalytic activity isolated~~ by steps comprising:

a) preparation of the immunoglobulin VL and VH cDNA by reverse-transcriptase polymerase chain reaction;

b) cloning the VL and VH cDNA in a vector in a form enabling their expression as single chain Fv fragments expressed on the surface of a display vector; and

c) contacting the vector particles with inmmobilized pCRA of claim 1, removal of unbound vector particles by washing, and expression of the Fv genes from the pCRA-bound vector particles in soluble form in prokaryotic or eukaryotic cells. [[;]]

~~d) screening the soluble Fv constructs for covalent antigen binding activity;~~

~~e) screening the soluble Fv constructs for catalytic activity.~~

Claim 19 (original): The method of claim 12, wherein lymphocytes are obtained by steps comprising:

a) contacting the lymphocytes with a pCRA;

b) separating lymphocytes that are bound to the pCRA from lymphocytes that are not bound to the pCRA.

Claim 20 (canceled).

Claim 21 (original): The method of claim 1, wherein the antibodies belong to the IgG, IgM, IgD, IgA or IgE classes.

Claim 22 (original): The method of claim 1, wherein the antibodies are fragments of IgG, IgM, IgD, IgA or IgE.

Claim 23 (original): The method of claim 1, wherein [L_1 . . . Lx . . . Lm] represents an antigenic determinant of a microbial protein.

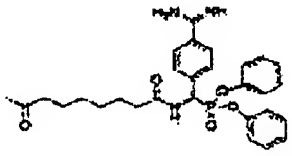
Claim 24 (canceled).

Claim 25 (original): The method of claim 1, wherein [L_1 . . . Lx . . . Lm] represents an antigenic determinant of a human, animal or plant protein.

Claims 26-28 (canceled).

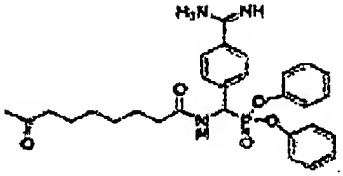
Claim 29 (original): The method of claim 1, wherein n is from 1 to 23.

Claim 30 (original): The method of claim 1, wherein the pCRA is gp120 derivatized at the Lys side chain amino groups at a density of 23 moles/mole protein with:

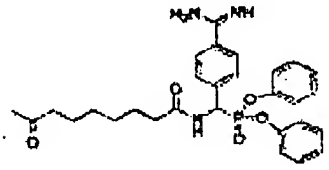


Claim 31 (canceled).

Claim 32 (original): The method of claim 1, wherein the pCRA is vasoactive intestinal peptide derivatized at the Lys20 side chain with:



Claim 33 (currently amended): The method of claim 1, wherein the antigenic ~~immunogenic~~ determinant is derived from the soluble extra-cellular domain of epidermal growth factor receptor, soluble extra-cellular domain of CD4, Factor VIII, .beta.-amyloid peptide 1-40 or .beta.-amyloid peptide 1-42, each derivatized at Lys side chains with:



Claim 34 (withdrawn and currently amended): The method of claim 12, wherein the [[M]]monoclonal IgG antibody clones YZ-18, YZ-20 and YZ-24 that catalyze the cleavage of gp120.

Claim 35 (withdrawn and currently amended): The method of claim 12, wherein the [[M]]monoclonal IgG antibody clones YZ-18, YZ-19, YZ-20, YZ-21, YZ-22, YZ-23 and YZ-24 that bind the gp120-CRA of claim 30 and the binding is resistant to dissociation with 2% SDS.

Claim 36 (withdrawn and currently amended): The method of claim 12, wherein the [[M]]monoclonal IgG antibody clones YZ-18, YZ-19, YZ-20, YZ-21, YZ-22, YZ-23 and YZ-24 that bind gp120 and the binding is resistant to dissociation with 2% SDS.

Claim 37 (withdrawn and currently amended): The method of claim 12, wherein [[F]]full-length IgG, IgM and IgA antibodies are prepared from the antibody fragments ~~of claim 12, prepared~~ by steps comprising:

a) insertion of the VL and VH domain cDNA at the 5' side of Ig constant domains contained in an expression vector by nucleic acid digestion and ligation procedures;

b) growth of the vector in a prokaryotic or eukaryotic host cell, extraction of the full-length antibodies from the culture medium or the cellular contents and purification of said antibodies.

Claim 38 (withdrawn and currently amended): A method of obtaining monoclonal covalent antibodies, catalytic antibodies, covalent antibody fragments or catalytic antibody fragments from the lymphocytes of organisms with autoimmune disease, organisms with autoimmune disease, organisms without known disease or ~~transenic~~ transgenic mice expressing human antibody genes comprising the steps:

a) preparing a library of hybridoma cell lines, virus-transformed cell lines or immunoglobulin fragment genes cloned in and expressed from a vector;

b) screening and selection for covalent activity of antibodies or antibody fragments by binding to an antigenic pCRA of claim 1 or a polypeptide;

c) screening and selection for catalytic hydrolysis of a polypeptide by the antibodies or antibody fragments; and

d) purifying the antibodies or the antibody fragments.

Claim 39 (canceled).

Claim 40 (withdrawn): The method of claim 38, wherein the antibodies hydrolyze peptide bonds in superantigenic polypeptides.

Claim 41 (withdrawn): The method of claim 38, wherein the antibodies hydrolyze gp120.

Claim 42 (withdrawn): The method of claim 38, wherein the antibodies hydrolyze CD4.

Claim 43 (withdrawn): The method of claim 38, wherein the antibodies hydrolyze .beta.-amyloid peptides.

Claim 44 (canceled).

Claim 45 (withdrawn): The method of claim 38, wherein the autoimmune disease is systemic lupus erythematosus.

Claim 46 (withdrawn): The method of claim 38, wherein the immunoglobulin fragments are the VL and VH domains linked by a peptide linker.

Claim 47 (withdrawn): The method of claim 38, wherein the immunoglobulin fragments are the light chain subunits.

Claim 48 (withdrawn): The method of claim 38, wherein the vector is selected from the group consisting of phage display vectors, retroviral display vectors, yeast display vectors, bacterial display vectors and mammalian display vectors.

Claim 49 (canceled).

Claim 50 (withdrawn): The method of claim 38, wherein the antibody fragments are single chain Fv fragments or light chains expressing covalent or catalytic activity isolated by steps comprising:

a) preparing the immunoglobulin VL cDNA, VH cDNA and light chain cDNA by reverse-transcriptase polymerase chain reaction using as template the RNA from lymphocytes;

b) cloning the VL and VH cDNA in a form enabling their expression as single chain Fv fragments expressed on the surface of a display vector;

c) cloning the light chain cDNA in a vector in a form enabling their expression as light chains expressed on the surface of a display vector;

d) contacting the vector particles with immobilized pCRA of claim 1, removal of unbound vector particles by washing, and expressing the Fv cDNA or light chain cDNA from the pCRA-bound vector particles in soluble form in prokaryotic or eukaryotic cells;

e) screening the soluble Fv or light chain constructs for covalent antigen binding activity;

f) screening the soluble Fv or light chain constructs for catalytic activity.

Claim 51 (withdrawn and currently amended): Full-length IgG, IgM and IgA antibodies prepared from the Fv fragments of claim 50 ~~38~~ prepared by steps comprising:

a) insertion of the VL and VH: domain cDNA at the 5' side of Ig constant domains contained in an expression vector by nucleic acid digestion and ligation procedures;

b) growth of the vectors in a prokaryotic or eukaryotic host cell, extraction of the full-length antibodies from the culture medium or the cellular contents and purification of said antibodies.

Claim 52 (withdrawn and currently amended): Full-length IgG, IgM and IgA antibodies prepared from the light chain fragments of claim 50 ~~38~~ prepared by steps comprising:

a) insertion of the light chain cDNA into an expression vector by nucleic acid digestion and ligation procedures;

b) insertion of the VH domain of gp120 binding antibodies at the 5' side of an IgG heavy chain constant domain contained in an expression vector by nucleic acid digestion and ligation procedures;

c) growth of the vectors in a prokaryotic or eukaryotic host cell, extraction of the full-length antibodies from the culture medium or the cellular contents and purification of said antibodies.

Claim 53 (withdrawn): The method of claim 38, wherein lymphocytes are obtained by steps comprising:

a) contacting the lymphocytes with a pCRA;

b) separating lymphocytes that are bound to the pCRA from lymphocytes that are not bound to the pCRA.

Claim 54 (canceled).

Claim 55 (withdrawn): The method of claim 38, wherein the antibodies belong to the IgG, IgM, IgD, IgA or IgE classes.

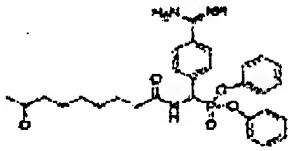
Claim 56 (withdrawn): The method of claim 38, wherein [L_1 . . . Lx . . . Lm] in the pCRA represents an antigenic determinant of a microbial protein.

Claim 57 (withdrawn): The method of claim 38, wherein [L_1 . . . Lx . . . Lm] in the pCRA represents an antigenic determinant of the HIV-1 protein.gp120.

Claims 58-61 (canceled).

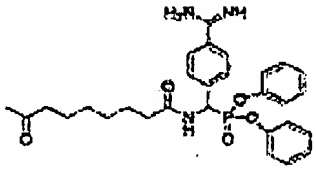
Claim 62 (withdrawn): The method of claim 38, wherein n is from 1 to 23.

Claim 63 (withdrawn): The method of claim 38, wherein the pCRA is gp120 derivatized at the Lys side chain amino groups at a density of 23 moles/mole protein with:

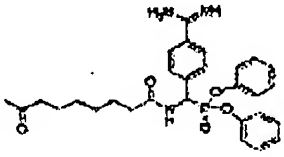


Claim 64 (canceled).

Claim 65 (withdrawn): The method of claim 38, wherein the pCRA is vasoactive intestinal peptide derivatized at the Lys20 side chain with:



Claim 66 (withdrawn): The method of claim 38, wherein the immunogenic determinant is derived from the soluble extra-cellular domain of the epidermal growth factor receptor, soluble extra-cellular domain of CD4, Factor VIII, .beta.-amyloid peptide 1-40 or .beta.-amyloid peptide 1-42, each derivatized at Lys side chains with:



Claim 67 (withdrawn): A method to improve the covalent or catalytic activity of the antibody fragments of claim 12, comprising the steps:

a) introducing mutations in the VL and VH domains;

b) display of the resultant antibody fragments on the surface of a display vector;

c) contacting the vector particles with the pCRAW, and removal of unbound vector particles

d) expressing the antibody fragments in soluble form in prokaryotic or eukaryotic cells;

d) screening the antibody fragments for covalent antigen binding activity;

e) screening the antibody fragments for catalytic activity.

Claim 68 (canceled).

Claim 69 (withdrawn): A method for passive immunotherapy of a disease, comprising:

a) administering a therapeutically effective amount of antibodies having covalent or catalytic activity specific for an antigen associated with a medical disorder in the patient, said antibody having been produced by the method of claim 1; and

b) repeating step a) as necessary for maintenance therapy.

Claim 70 (withdrawn): A method for passive immunotherapy of a disease, comprising:

a) administering a therapeutically effective amount of antibodies having covalent or catalytic activity specific for an antigen associated with a medical disorder in the patient, said antibody having been produced by the method of claim 38; and

b) repeating step a) as necessary for maintenance therapy.

Claim 71 (original): The method of claim 1, wherein the antibody is directed to gp120 for immunotherapy of HIV-1 infection.

Claims 72-75 (canceled).

Claim 76 (withdrawn): A method for stimulating production of prophylactic antibodies in an organism, having covalent or catalytic activity specific for an antigen associated with a medical condition in the organism, comprising the steps of:

a) administering to an organism a vaccine containing an immunogenic amount of a pCRA prepared from said antigen as of claim 1;

b) repeating step a) as necessary to ensure effective antibody production.

Claim 77 (withdrawn): The method of claim 76, in which the medical disorder is a microbial disease and the pCRA is prepared from a constituent protein of the microbe.

Claim 78 (withdrawn): The method of claim 77, in which the medical disorder is HIV-1 infection and the pCRA is prepared from gp120.

Claim 79 (withdrawn): A method of treating a medical disorder in a patient by inhibiting the action of a catalytic antibody, comprising the steps of:

a) administering to said patient a therapeutic amount of a pCRA in which the antigenic determinant is derived from an epitope irreversibly bound by said catalytic antibody;

b) assessing said patient for inactivation of said catalytic antibody; and

c) repeating step a) as necessary to maintain inhibition of said action of said catalytic antibody.

Claim 80 (withdrawn): The method of claim 79, wherein said disease state is an autoimmune disease.

Claim 81 (canceled).

Claim 82 (withdrawn): The method of claim 79, wherein said medical disorder is a lymphoproliferative disorder.

Claim 83 (withdrawn): The method of claim 82, wherein said lymphoproliferative disorder is selected from the group consisting of multiple mycloma, acute lymphoblastic leukemia, lymphoblastic lymphoma, small lymphocytic lymphoma, lymphoplasmacytoid lymphoma, Waldenstroms macroglobulinemia, follicular center lymphoma, mucosa-associated lymphoid tissue lymphoma, hairy cell leukemia, diffuse large B-cell lymphoma, Burkitts lymphoma, and node based moncocytoid lymphoma.

Claim 84 (withdrawn): The method of claim 12, wherein the organism expresses a genetic defect resulting in defective B cell receptor mediated transmembrane signaling in B cells.

Claim 85 (withdrawn): The method in claim 84, in which the defective B cell receptor mediated transmembrane signaling is caused by altered expression of CD19, CD22 or Lyn.

Claim 86 (new). The method of claim 1, wherein Y", Y' or Y contains a water-binding group as a terminal or internal component.

Claim 87 (new): The method of claim 86, wherein the water-binding group is composed of a site that binds a metal ion which chelates one or more water molecules.

Claim 88 (new): The method of claim 87, in which the metal is zinc, copper, nickel, cobalt, calcium or magnesium.

Claim 89 (new): The method of claim 87, in which the metal binding group is selected from: -(His),sub,n- where n=2 or more, -Cys-X-Cys-Cys- or -Cys-X-Cys- wherein X is an amino acid residue, ethylene diamine tetraacetic acid or diaminomethyl pyridine.